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(54) Title: HORMONE-SENSITIVE LIPASE MEDIATED MALE INFERTILITY

(57) Abstract: In various aspects, the invention provides pharmacologic and genomic methods of inhibiting fertility in a male animal, such as human male contraception, involving the manipulation of the activity of hormone-sensitive lipase, including inhibiting the activity of a hormone-sensitive lipase in the animal. In another aspect, the invention discloses the use of hormone-sensitive lipase as a target in screening assays that may be used to identify compounds that modulate male fertility. In another aspect, the present invention identifies a condition of male infertility caused by hormone-sensitive lipase deficiency.

HORMONE-SENSITIVE LIPASE MEDIATED MALE INFERTILITY

FIELD OF THE INVENTION

The invention is in the field of pharmacologic and genomic methods of male contraception, involving the manipulation of the testicular activity of hormone-sensitive lipase.

BACKGROUND OF THE INVENTION

There is a continuing need for methods of modulating male fertility. For example, in humans, there is a need for alternatives to surgical approaches to male contraception such as castration and vasectomy. There is also a new and growing need for methods of inhibiting fertility in genetically modified organisms, as a means for preventing the dissemination of genetic modifications into wild-type populations.

Hormone-sensitive lipase (E.C. 3.1.1.3) has several substrates, all of which are fatty acyl esters. Hormone-sensitive lipase cleaves fatty acids from triacylglycerides and diacylglycerides at the 1- and 3-positions, cholesteryl esters and esters of retinoic acid and of steroid hormones. Hormone-sensitive lipase is active in adipose tissue, where it plays an important role in the release of fatty acids (Langin *et al.*, 1996, *Proceeding of the Nutrition Society* 55:93). Hormone-sensitive lipase is also expressed in many non-adipose tissues, such as adrenal gland, pancreatic beta cells, macrophages and testicles (Holst *et al.*, 1996, *Genomics* 35:441; Mulder *et al.*, 1999, *Diabestes* 48:228; Reue *et al.*, 1997, *Arterioscler Thromb Basic Biol* 17:3428; Blaise *et al.*, 1999, *J. Biol. Chem* 274:9327).

The human hormone-sensitive lipase gene has been cloned (Langin *et al.*, 1993, *Proc. Natl. Acad. Sci USA* 90:4897). Hormone-sensitive lipase is an amphiphilic 84 Kda protein with little homology to other mammalian lipases, containing three residues thought to be essential for serine protease activity (Ser 424, Asp 693 and His 723). In testes, hormone-sensitive lipase is expressed as a 130 KDa isoform, for which transcription begins in a specific upstream exon that is spliced to a site 22 base pairs upstream of the initiation methionine codon of nontesticular transcripts. The testicular hormone-sensitive lipase isoform therefore contains all of the sequence of nontesticular hormone-sensitive lipase plus

an N-terminal extension encoded by the first (testes specific) exon. The testicular form of hormone-sensitive lipase is expressed at a specific phase of sperm development, in round and elongating spermatids.

A number of small molecule inhibitors of hormone-sensitive lipase are known, as shown in Table 1.

Table 1: Inhibitors of hormone-sensitive lipase

Inhibitor	IC50*
Diisopropylfluorophosphate (DFP)	9 microM
$HgCl_2$ (mercuric chloride)	11 microM
NaF (sodium fluoride)	25 Mm

* concentration producing 50% inhibition (Stralfors *et al.*, 1987, The Enzymes, Vol. XVIII, p.147-177, Academic Press)

SUMMARY OF THE INVENTION

In various aspects, the invention provides pharmacologic and genomic methods of inhibiting fertility in a male animal, such as human male contraception, involving the manipulation of the activity of hormone-sensitive lipase, including inhibiting the activity of a hormone-sensitive lipase in the animal. In the context of the invention, inhibiting the activity of hormone-sensitive lipase includes reducing the activity of hormone-sensitive lipase by suppression of enzymatic activity or suppression of transcription or translation of hormone-sensitive lipase. In one aspect, genomic manipulations may be used to affect the expression of hormone-sensitive lipase, or to affect the activity of the expressed enzyme. In another aspect, pharmaceuticals may be administered to modulate the activity of hormone-sensitive lipase. In some aspects of the invention, a testicular isoform of hormone-sensitive lipase may be the target of pharmacologic or genomic modulation. In some aspects, the invention provides methods of down-regulating testicular hormone-sensitive lipase to mediate male infertility by effecting the spermiogenesis phase of spermatogenesis. The method may for example comprise administering to the male animal an effective amount of a hormone-sensitive lipase inhibitor, which constitutes a use of a hormone-sensitive lipase inhibitor to inhibit fertility in a male animal.

In another aspect, the invention discloses the use of hormone-sensitive lipase as a target in screening assays that may be used to identify compounds that modulate male fertility. Such assays may be utilized to identify compounds that modulate expression of the hormone-sensitive lipase gene, or compounds that modulate the activity of the expressed enzyme. The testicular isoform of hormone-sensitive lipase may be used as the target in such assays. The use of a hormone-sensitive lipase in an assay to screen for a compound that inhibits male fertility may involve a method for identifying male fertility inhibitors comprising:

a) providing a test compound;

b) providing a hormone-sensitive lipase;

c) providing a substrate for the hormone-sensitive lipase;

d) assaying the activity of the hormone-sensitive lipase on the substrate in the presence of the test compound, to identify test compounds that inhibit the hormone-sensitive lipase.

The method may further comprise the step of assaying the compounds for inhibition of male fertility, for example by inhibiting spermatogenesis.

In another aspect, the present invention identifies a condition of male infertility caused by hormone-sensitive lipase deficiency. A screening method is therefore provided to provide information about testicular hormone-sensitive lipase activity in males. Such screening methods may be genomic or enzymatic, to screen respectively for expression or activity of hormone-sensitive lipase. Such screening methods may be used to identify individuals having reduced testicular hormone-sensitive lipase activity. Accordingly, a method of screening male patients, may comprise:

a) identifying a male patient having reduced fertility;

b) assaying the activity of a testicular hormone-sensitive lipase in the male patient.

DETAILED DESCRIPTION OF THE INVENTION

In one aspect of the invention, genomic manipulations may be used to affect the expression of hormone-sensitive lipase. Germ line transformation with a gene targeting vector may for example be used to disrupt expression of functional hormone-sensitive lipase,

as described in Example 1. A wide variety of alternative genomic approaches are available to down-regulate the expression of functional hormone-sensitive lipase. For example, in alternative embodiments, transformation of cells with antisense constructs may be used to inhibit expression of hormone-sensitive lipase. Antisense constructs are nucleic acid molecules that may be transcribed to provide an antisense molecule that is substantially complementary to all or a portion of the mRNA encoding a hormone-sensitive lipase, so that expression of the antisense construct interferes with the expression of the hormone-sensitive lipase. SEQ ID NO:1 is for example a putative human mRNA encoding the testicular isoform of hormone-sensitive lipase, with the putative coding sequence shown in SEQ ID NO:1 and the protein sequence shown as SEQ ID NO: 2 (from Holst *et al.*, 1995, GenBank Accession No. NM_005357; see also Holst *et al.*, 1996, *Genomics* 35:441 and related GenBank Accession Nos. U40001 and U40002). In some embodiments, antisense constructs of the invention may therefore encode five or more contiguous nucleic acid residues substantially complimentary to a contiguous portion a nucleic acid sequence encoding a hormone-sensitive lipase, such as SEQ ID NO:1 or another mRNA encoding a mammalian hormone-sensitive lipase. In one aspect of the invention, antisense constructs may be provided that encode a nucleic acid that is complementary to a testis-specific portion of a hormone-sensitive lipase mRNA, such as the testis-specific exon of the human testicular isoform of hormone-sensitive lipase (Holst *et al.*, 1996, *Genomics* 35:441).

Substantially complementary nucleic acids are nucleic acids in which the complement of one molecule is substantially identical to the other molecule. Two nucleic acid or protein sequences are considered substantially identical if, when optimally aligned, they share at least about 70% sequence identity. In alternative embodiments, sequence identity may for example be at least 75%, at least 90% or at least 95%. Optimal alignment of sequences for comparisons of identity may be conducted using a variety of algorithms, such as the local homology algorithm of Smith and Waterman,1981, *Adv. Appl. Math* 2: 482, the homology alignment algorithm of Needleman and Wunsch, 1970, *J. Mol. Biol.* 48:443, the search for similarity method of Pearson and Lipman, 1988, *Proc. Natl. Acad. Sci. USA* 85: 2444, and the computerised implementations of these algorithms (such as GAP, BESTFIT, FASTA and TFASTA in the Wisconsin Genetics Software Package, Genetics Computer Group, Madison, WI, U.S.A.). Sequence identity may also be determined using the BLAST algorithm,

described in Altschul *et al.*, 1990, *J. Mol. Biol.* 215:403-10 (using the published default settings). Software for performing BLAST analysis may be available through the National Center for Biotechnology Information (through the internet at http://www.ncbi.nlm.nih.gov/). The BLAST algorithm involves first identifying high scoring sequence pairs (HSPs) by identifying short words of length W in the query sequence that either match or satisfy some positive-valued threshold score T when aligned with a word of the same length in a database sequence. T is referred to as the neighbourhood word score threshold. Initial neighbourhood word hits act as seeds for initiating searches to find longer HSPs. The word hits are extended in both directions along each sequence for as far as the cumulative alignment score can be increased. Extension of the word hits in each direction is halted when the following parameters are met: the cumulative alignment score falls off by the quantity X from its maximum achieved value; the cumulative score goes to zero or below, due to the accumulation of one or more negative-scoring residue alignments; or the end of either sequence is reached. The BLAST algorithm parameters W, T and X determine the sensitivity and speed of the alignment. The BLAST program may use as defaults a word length (W) of 11, the BLOSUM62 scoring matrix (Henikoff and Henikoff, 1992, *Proc. Natl. Acad. Sci. USA* 89: 10915-10919) alignments (B) of 50, expectation (E) of 10 (or 1 or 0.1 or 0.01 or 0.001 or 0.0001), M=5, N=4, and a comparison of both strands. One measure of the statistical similarity between two sequences using the BLAST algorithm is the smallest sum probability (P(N)), which provides an indication of the probability by which a match between two nucleotide or amino acid sequences would occur by chance. In alternative embodiments of the invention, nucleotide or amino acid sequences are considered substantially identical if the smallest sum probability in a comparison of the test sequences is less than about 1, preferably less than about 0.1, more preferably less than about 0.01, and most preferably less than about 0.001.

An alternative indication that two nucleic acid sequences are substantially complementary is that the two sequences hybridize to each other under moderately stringent, or preferably stringent, conditions. Hybridisation to filter-bound sequences under moderately stringent conditions may, for example, be performed in 0.5 M $NaHPO_4$, 7% sodium dodecyl sulfate (SDS), 1 mM EDTA at 65EC, and washing in 0.2 x SSC/0.1% SDS at 42EC (see Ausubel, *et al.* (eds), 1989, *Current Protocols in Molecular Biology*, Vol. 1, Green

Publishing Associates, Inc., and John Wiley & Sons, Inc., New York, at p. 2.10.3). Alternatively, hybridization to filter-bound sequences under stringent conditions may, for example, be performed in 0.5 M $NaHPO_4$, 7% SDS, 1 mM EDTA at 65EC, and washing in 0.1 x SSC/0.1% SDS at 68EC (see Ausubel, *et al.* (eds), 1989, *supra*). Hybridization conditions may be modified in accordance with known methods depending on the sequence of interest (see Tijssen, 1993, *Laboratory Techniques in Biochemistry and Molecular Biology -- Hybridization with Nucleic Acid Probes*, Part I, Chapter 2 "Overview of principles of hybridization and the strategy of nucleic acid probe assays", Elsevier, New York). Generally, stringent conditions are selected to be about 5EC lower than the thermal melting point for the specific sequence at a defined ionic strength and pH.

In alternative embodiments, the invention provides antisense molecules and ribozymes for exogenous administration to bind to, degrade and/or inhibit the translation of hormone-sensitive lipase mRNA. Examples of therapeutic antisense oligonucleotide applications, incorporated herein by reference, include: U.S. Pat. No. 5,135,917, issued Aug. 4, 1992; U.S. Pat. No. 5,098,890, issued Mar. 24, 1992; U.S. Pat. No. 5,087,617, issued Feb. 11, 1992; U.S. Pat. No. 5,166,195 issued Nov. 24, 1992; U.S. Pat. No. 5,004,810, issued Apr. 2, 1991; U.S. Pat. No. 5,194,428, issued Mar. 16, 1993; U.S. Pat. No. 4,806,463, issued Feb. 21, 1989; U.S. Pat. No. 5,286,717 issued Feb. 15, 1994; U.S. Pat. No. 5,276,019 and U.S. Pat. No. 5,264,423; BioWorld Today, Apr. 29, 1994, p. 3.

Preferably, in antisense molecules, there is a sufficient degree of complementarity to the hormone-sensitive lipase ~~mRNA to avoid~~ non-specific binding of the antisense molecule to non-target sequences under conditions in which specific binding is desired, such as under physiological conditions in the case of in vivo assays or therapeutic treatment or, in the case of in vitro assays, under conditions in which the assays are conducted. The target mRNA for antisense binding may include not only the information to encode a protein, but also associated ribonucleotides, which for example form the 5'-untranslated region, the 3'-untranslated region, the 5' cap region and intron/exon junction ribonucleotides. A method of screening for antisense and ribozyme nucleic acids that may be used to provide such molecules as hormone-sensitive lipase inhibitors of the invention is disclosed in U.S. Patent No. 5,932,435 (which is incorporated herein by reference).

Antisense molecules (oligonucleotides) of the invention may include those which contain intersugar backbone linkages such as phosphotriesters, methyl phosphonates, short chain alkyl or cycloalkyl intersugar linkages or short chain heteroatomic or heterocyclic intersugar linkages, phosphorothioates and those with CH_2--NH--O--CH_2, CH_2--N(CH_3)--O--CH_2 (known as methylene(methylimino) or MMI backbone), CH_2--O--N(CH_3)--CH_2, CH_2--N(CH_3)--N(CH_3)--CH_2 and O--N(CH_3)--CH_2 --CH_2 backbones (where phosphodiester is O--P--O--CH_2). Oligonucleotides having morpholino backbone structures may also be used (U.S. Pat. No. 5,034,506). In alternative embodiments, antisense oligonucleotides may have a peptide nucleic acid (PNA, sometimes referred to as "protein nucleic acid") backbone, in which the phosphodiester backbone of the oligonucleotide may be replaced with a polyamide backbone wherein nucleosidic bases are bound directly or indirectly to aza nitrogen atoms or methylene groups in the polyamide backbone (Nielsen *et al.*, 1991, Science 254:1497 and U.S. Pat. No. 5,539,082). The phosphodiester bonds may be substituted with structures which are chiral and enantiomerically specific. Persons of ordinary skill in the art will be able to select other linkages for use in practice of the invention.

Oligonucleotides may also include species which include at least one modified nucleotide base. Thus, purines and pyrimidines other than those normally found in nature may be used. Similarly, modifications on the pentofuranosyl portion of the nucleotide subunits may also be effected. Examples of such modifications are 2'-O-alkyl- and 2'-halogen-substituted nucleotides. Some specific examples of modifications at the 2' position of sugar moieties which are useful in the present invention are OH, SH, SCH_3, F, OCN, $O(CH_2)_n NH_2$ or $O(CH_2)_n CH_3$ where n is from 1 to about 10; C_1 to C_{10} lower alkyl, substituted lower alkyl, alkaryl or aralkyl; Cl; Br; CN; CF_3 ; OCF_3 ; O-, S-, or N-alkyl; O-, S-, or N-alkenyl; $SOCH_3$; $SO_2 CH_3$; ONO_2 ; NO_2 ; N_3; NH_2; heterocycloalkyl; heterocycloalkaryl; aminoalkylamino; polyalkylamino; substituted silyl; an RNA cleaving group; a reporter group; an intercalator; a group for improving the pharmacokinetic properties of an oligonucleotide; or a group for improving the pharmacodynamic properties of an oligonucleotide and other substituents having similar properties. One or more pentofuranosyl groups may be replaced by another sugar, by a sugar mimic such as cyclobutyl or by another moiety which takes the place of the sugar.

In some embodiments, the antisense oligonucleotides in accordance with this invention may comprise from about 5 to about 100 nucleotide units. As will be appreciated, a nucleotide unit is a base-sugar combination (or a combination of analogous structures) suitably bound to an adjacent nucleotide unit through phosphodiester or other bonds forming a backbone structure.

In another aspect, pharmaceuticals may be administered to modulate the activity of hormone-sensitive lipase. In this aspect of the invention, inhibitors of hormone-sensitive lipase may be selected from the group consisting of diisopropylfluorophosphate, mercuric chloride ($HgCl_2$) and sodium fluoride (NaF). In some aspects of the invention, a testicular isoform of hormone-sensitive lipase may be the target of pharmacologic or genomic modulation. For example, controlled-release formulations of hormone-sensitive lipase inhibitors may be administered to the testis or testicular region. In some aspects, the invention accordingly provides methods of down-regulating testicular hormone-sensitive lipase to mediate male infertility by affecting the spermiogenesis phase of spermatogenesis.

In another aspect, the invention discloses the use of hormone-sensitive lipase as a target in screening assays that may be used to identify compounds that modulate male fertility. In some embodiments, such an assay may comprise the steps of

a) providing a test compound;

b) providing a hormone-sensitive lipase;

c) providing a substrate for the hormone-sensitive lipase;

d) assaying the activity of the hormone-sensitive lipase on the substrate in the presence of the compound, to identify compounds that inhibit the hormone-sensitive lipase.

Such an assay may further comprise the step of assaying the compounds for spermatogenesis or spermiogenesis inhibiting activity. Such assays may be utilized to identify compounds that modulate expression of the hormone-sensitive lipase gene, or compounds that modulate the activity of the expressed enzyme. The testicular isoform of hormone-sensitive lipase may be used as the target in such assays.

In one embodiment, screening assays of the invention may comprise identifying compounds that modulate the cholesteryl esterase activity of hormone-sensitive lipase. For

example, the neutral cholesteryl esterase activity of hormone-sensitive lipase may be assayed (Kraemer *et al.*, 1993, *J. Lipid Res.* 34:663). In alternative embodiments, lipolysis may be measured (Susulic *et al.*, 1995, *J. Biol. Chem.* 270:29483) with or without the addition of a lipolytic beta-3 adrenergic agonist, such as 10μM CL316,243 (Wyeth-Ayerst Research Laboratories, Princeton, NJ, USA).

In another aspect, the present invention identifies a condition of male infertility caused by hormone-sensitive lipase deficiency. A screening method is therefore provided to provide information about testicular hormone-sensitive lipase activity in males. Such screening methods may be genomic or enzymatic, to screen respectively for expression or activity of hormone-sensitive lipase. Such methods are disclosed in Example 1. Such screening methods may be used to identify individuals having reduced testicular hormone-sensitive lipase activity.

Although various embodiments of the invention are disclosed herein, many adaptations and modifications may be made within the scope of the invention in accordance with the common general knowledge of those skilled in this art. Such modifications include the substitution of known equivalents for any aspect of the invention in order to achieve the same result in substantially the same way. Numeric ranges are inclusive of the numbers defining the range. In the claims, the word "comprising" is used as an open-ended term, substantially equivalent to the phrase "including, but not limited to". The following examples are illustrative of various aspects of the invention, and do not limit the broad aspects of the invention as disclosed herein.

Example 1

Murine transformation by blastocyst microinjection of a gene targeting vector was used to create chimeric mice having a functionally defective hormone-sensitive lipase allele. The mutant hormone-sensitive lipase allele lacked codons 2-172. The targeting vector was constructed from an *Af*II/*Bam*HI subclone of a murine genomic hormone-sensitive lipase clone (Sztrolovics *et al.*, 1997, *Mamm Genome* 8:86). In the mutant allele, 513 coding base pairs in exon 1 and 1,494 base pairs in intron 1 were replaced with a promoterless beta galactosidase gene inserted in frame with the hormone-sensitive lipase coding sequences,

with a neomycin resistance cassette having a herpes simplex thymidine kinase promoter (pMC1Neo poly A, Stratagene).

Heterozygous F1 offspring of a male chimera with the mutant hormone-sensitive lipase allele and Balb/c females were crossed to produce mice for analysis. Germline transmission of the allele was obtained, and mice homozygous for the allele were produced.

Male hormone-sensitive-lipase-deficient mice having the defective hormone-sensitive lipase allele were completely infertile. Fertility was assessed by housing two month old males for 5 days with two CD1 females, then separating the females for 5 days before rebreeding. Females not becoming pregnant were shown to be fertile by subsequent mating with controls. Wild-type mice from the same litter had normal fertility. Mating occurred at a similar rate in the wild-type and hormone-sensitive-lipase-deficient mice, as indicated by the presence of mucus plugs in females that were housed with the mice. Seminal vesicles from the hormone-sensitive-lipase-deficient mice were of normal weight and histology, suggesting that these mice are not androgen deficient. Testicles from mutant mice weighed about half as much as those from normal mice, this is consistent with a lack of spermatozoa since spermatozoa comprise about half of normal testicular weight. Histology confirmed this, revealing an absence of spermatozoa in the seminal vesicles and epidiymis (i.e. azoospermia). Spermatogenesis was similar in hormone-sensitive-lipase-deficient and wild-type mice during the development of mitotic (spermatogonia) and meiotic (spermatocyte) germ cells. In postmeiotic development (spermiogenesis) marked abnormalities were noted in the germ cells of hormone-sensitive-lipase-deficient mice, including the presence of large cytoplasmic masses near the tubular lumen, multi-nucleated spermatids, some sharing a common acrosome, disruption of the normal orientation of the heads of late spermatids, and by stage VII of the seminiferous epithelial cycle a striking decrease in the number of late spermatids

Southern blot analysis showed that the hormone-sensitive-lipase-deficient allele was present in the transformed mice. Northern analysis (Wang *et al.*, 1993, *Mamm Genome* 4:382) using mouse cDNA probes from residues of 545-869 of the hormone-sensitive lipase gene showed that no normal hormone-sensitive lipase mRNA was expressed in these mice.

Cholesteryl esterase activity was undetectable in adipose tissue of mutant mice, consistent with a lack of functional hormone-sensitive lipase. Cholesteryl esterase activity was assayed as neutral cholesteryl esterase activity (Kraemer *et al.*, 1993, *J. Lipid Res.* 34:663). Perigonadal fat pad adipocytes were isolated (Shillabeer and Lau, 1994, *J. Lipid Res.* 35:592) and lipolysis measured (Susulic *et al.*, 1995, *J. Biol. Chem.* 270:29483) with or without the addition of 10μM lipolytic beta-3 adrenergic agonist CL316,243 (Wyeth-Ayerst Research Laboratories, Princeton, NJ, USA). For each mixture, triglyceride concentration was assayed, the diameters of 200 adipocytes were measured following fixation in 4% glutaraldehyde and the mean adipocyte lipid content, content and surface area calculated.

Six-month-old hormone-sensitive-lipase-deficient mice were grossly normal and similar to wild-type littermates in weight and adipose tissue histology. Plasma triglyceride and nonesterified fatty acids were lower in hormone-sensitive-lipase-deficient males. The mean diameter of adipocytes isolated from perigonadal fat pads of six-month old mice was smaller in hormone-sensitive-lipase-deficient mice than in wild-type mice. When corrected for cell surface area, basal lipolysis in mutant adipocytes exceeded that of controls. Hormone-sensitive-lipase-deficient adipocytes lacked adrenergic responsiveness, in contrast to a 4- to 9-fold increase in wild-type adipocytes.

Example 2: Therapeutic Formulations

In various embodiments, hormone-sensitive lipase inhibitors may be used therapeutically in formulations or medicaments to inhibit male fertility. The invention provides corresponding methods of medical treatment, in which a therapeutic dose of a hormone-sensitive lipase inhibitor is administered in a pharmacologically acceptable formulation. Accordingly, the invention also provides therapeutic compositions comprising an hormone-sensitive lipase inhibitor and a pharmacologically acceptable excipient or carrier. The therapeutic composition may be soluble in an aqueous solution at a physiologically acceptable pH.

The invention provides pharmaceutical compositions (medicaments) containing (comprising) hormone-sensitive lipase inhibitors. In one embodiment, such compositions include an hormone-sensitive lipase inhibitor in a therapeutically or prophylactically effective

amount sufficient to alter, and preferably inhibit, spermiogenesis, and a pharmaceutically acceptable carrier.

A "therapeutically effective amount" refers to an amount effective, at dosages and for periods of time necessary, to achieve the desired therapeutic result, such as reduction or reversal of spermiogenesis, or reduction or inhibition male fertility. A therapeutically effective amount of hormone-sensitive lipase inhibitor may vary according to factors such as the disease state, age, sex, and weight of the individual, and the ability of the compound to elicit a desired response in the individual. Dosage regimens may be adjusted to provide the optimum therapeutic response. A therapeutically effective amount is also one in which any toxic or detrimental effects of the compound are outweighed by the therapeutically beneficial effects. A "prophylactically effective amount" refers to an amount effective, at dosages and for periods of time necessary, to achieve the desired prophylactic result, such as preventing or inhibiting the rate of spermiogenesis or reducing male fertility. A prophylactically effective amount can be determined as described above for the therapeutically effective amount. For any particular subject, specific dosage regimens may be adjusted over time according to the individual need and the professional judgement of the person administering or supervising the administration of the compositions.

As used herein "pharmaceutically acceptable carrier" or "excipient" includes any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like that are physiologically compatible. In one embodiment, the carrier is suitable for parenteral administration. Alternatively, the carrier can be suitable for intravenous, intraperitoneal, intramuscular, sublingual or oral administration. Pharmaceutically acceptable carriers include sterile aqueous solutions or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersion. The use of such media and agents for pharmaceutically active substances is well known in the art. Except insofar as any conventional media or agent is incompatible with the active compound, use thereof in the pharmaceutical compositions of the invention is contemplated. Supplementary active compounds can also be incorporated into the compositions.

Therapeutic compositions typically must be sterile and stable under the conditions of manufacture and storage. The composition can be formulated as a solution, microemulsion, liposome, or other ordered structure suitable to high drug concentration. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyethylene glycol, and the like), and suitable mixtures thereof. The proper fluidity can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. In many cases, it will be preferable to include isotonic agents, for example, sugars, polyalcohols such as mannitol, sorbitol, or sodium chloride in the composition. Prolonged absorption of the injectable compositions can be brought about by including in the composition an agent which delays absorption, for example, monostearate salts and gelatin. Moreover, the hormone-sensitive lipase inhibitors can be administered in a time release formulation, for example in a composition which includes a slow release polymer. The active compounds can be prepared with carriers that will protect the compound against rapid release, such as a controlled release formulation, including implants and microencapsulated delivery systems. Biodegradable, biocompatible polymers can be used, such as ethylene vinyl acetate, polyanhydrides, polyglycolic acid, collagen, polyorthoesters, polylactic acid and polylactic, polyglycolic copolymers (PLG). Many methods for the preparation of such formulations are patented or generally known to those skilled in the art.

Sterile injectable solutions can be prepared by incorporating the active compound (e.g. hormone-sensitive lipase inhibitor) in the required amount in an appropriate solvent with one or a combination of ingredients enumerated above, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the active compound into a sterile vehicle which contains a basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum drying and freeze-drying which yields a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof. In accordance with an alternative aspect of the invention, an hormone-sensitive lipase inhibitor may be formulated with one or more additional compounds that enhance the solubility of the hormone-sensitive lipase inhibitor.

In accordance with another aspect of the invention, therapeutic compositions of the present invention, comprising a hormone-sensitive lipase inhibitor, may be provided in containers having labels that provide instructions for use of the hormone-sensitive lipase inhibitor to inhibit male fertility or inhibit spermiogenesis.

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WHAT IS CLAIMED IS:

1. A method of inhibiting fertility in a male animal comprising inhibiting the activity of a hormone-sensitive lipase in the animal.

2. The method of claim 1, wherein the hormone-sensitive lipase is a testicular hormone-sensitive lipase.

3. The method of claim 1 or 2, wherein the animal is a mammal.

4. The method of claim 1 or 2, wherein the animal is a human.

5. The method of claim 4, wherein the hormone-sensitive lipase has an amino acid sequence that is substantially identical to SEQ ID NO:2.

6. The method of claim 1, 2, 3, 4 or 5, wherein the method comprises inhibiting the expression of the hormone sensitive lipase.

7. The method of any one of claims 1 through 6, wherein the method comprises administering to the male animal an effective amount of a hormone-sensitive lipase inhibitor.

8. The method of claim 7, wherein the hormone-sensitive lipase inhibitor is an antisense molecule.

9. The method of claim 8 wherein the antisense molecule is a nucleic acid that is substantially complementary to a portion of an mRNA encoding a hormone-sensitive lipase.

10. The method of claim 9 wherein the mRNA is substantially identical to SEQ ID NO:1.

11. The method of claim 9 or 10 wherein the portion of the mRNA comprises at least 5 contiguous bases.

12. The use of a hormone-sensitive lipase in an assay to screen for a compound that inhibits male fertility.

13. The use of a hormone-sensitive lipase according to claim 12, wherein the hormone-sensitive lipase is a testicular isoform of hormone-sensitive lipase.

14. The use of the hormone-sensitive lipase according to claim 12 or 13, wherein the hormone-sensitive lipase is a human hormone-sensitive lipase.

15. The use of the hormone-sensitive lipase according to claim 12, wherein the hormone-sensitive lipase has an amino acid sequence substantially identical to SEQ ID NO:2.

16. A method for identifying male fertility inhibitors comprising:

a) providing a test compound;

b) providing a hormone-sensitive lipase;

c) providing a substrate for the hormone-sensitive lipase;

d) assaying the activity of the hormone-sensitive lipase on the substrate in the presence of the test compound, to identify test compounds that inhibit the hormone-sensitive lipase.

17. The method of claim 16, further comprising the step of assaying the compounds for spermatogenesis inhibiting activity.

18. The method of claim 16 or 17, wherein the hormone-sensitive lipase is a testicular hormone-sensitive lipase.

19. The method of claim 16, 17 or 18, wherein the hormone-sensitive lipase is a mammalian hormone-sensitive lipase.

20. The method of any one of claims 16 through 19, wherein the hormone-sensitive lipase is a human hormone-sensitive lipase.

21. The method of any one of claims 16 through 19, wherein the hormone-sensitive lipase has an amino acid sequence substantially identical to SEQ ID NO:2.

22. A method of screening male patients, comprising:

a) identifying a male patient having reduced fertility;

b) assaying the activity of a testicular hormone-sensitive lipase in the male patient.

23. The use of a hormone-sensitive lipase inhibitor to inhibit fertility in a male animal.

24. The use of a hormone-sensitive lipase inhibitor according to claim 23, wherein the hormone-sensitive lipase inhibitor is an antisense molecule.

25. The use of a hormone-sensitive lipase inhibitor according to claim 24 wherein the antisense molecule is a nucleic acid that is substantially complementary to a portion of an mRNA encoding a hormone-sensitive lipase.

26. The use of a hormone-sensitive lipase inhibitor according to claim 25 wherein the mRNA is substantially identical to SEQ ID NO:1.

27. The use of a hormone-sensitive lipase inhibitor according to claim 25 or 26 wherein the portion of the mRNA comprises at least 5 contiguous bases.

SEQUENCE LISTING

<110> HOPITAL SAINTE-JUSTINE

MITCHELL, Grant A.

WANG, Shu Pei

<120> HORMONE-SENSITIVE LIPASE MEDIATED MALE INFERTILITY

<130> 86081-2

<140> PCT/CA 00/01228

<141> 2000-10-13

<150> CA 2,286,451

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<170> PatentIn version 3.0

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ugaggaauca augagagauc ugugaagaga gagggcuggg ugggagccca gaaggauaga     240

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Ser Val Ser Arg Ser Asp Trp Gln Pro Glu Pro His Gln Arg Pro Ile
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acc ccg cua gag ccu ggg cca gaa aag aca ccc aua gcc cag cca gaa      391
Thr Pro Leu Glu Pro Gly Pro Glu Lys Thr Pro Ile Ala Gln Pro Glu
        25                  30                  35

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caa aga ccc cuc acc cag cag gag acc ccu gca caa cau gau gcu gaa      487
Gln Arg Pro Leu Thr Gln Gln Glu Thr Pro Ala Gln His Asp Ala Glu
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ucc cag aag gaa ccu aga gcc caa caa aaa ucu gcu uca caa gag gaa      535
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                75                  80                  85

uuu cuu gcc cca cag aag ccc gca cca cag caa uca ccu uac auc caa      583
Phe Leu Ala Pro Gln Lys Pro Ala Pro Gln Gln Ser Pro Tyr Ile Gln
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agg gug cug cuc acu caa cag gaa gcu gcc ucc cag cag gga ccu ggg      631
Arg Val Leu Leu Thr Gln Gln Glu Ala Ala Ser Gln Gln Gly Pro Gly
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cua gga aaa gaa ucu aua acu caa cag gag cca gca uug aga caa aga      679
Leu Gly Lys Glu Ser Ile Thr Gln Gln Glu Pro Ala Leu Arg Gln Arg
    120                 125                 130

cau gua gcc cag cca ggg ccu ggg cca gga gag cca ccu cca gcu caa      727
His Val Ala Gln Pro Gly Pro Gly Pro Gly Glu Pro Pro Pro Ala Gln
135                 140                 145                 150

caa gaa gcu gaa uca aca ccu gcg gcc cag gcu aaa ccu gga gcc aaa      775
Gln Glu Ala Glu Ser Thr Pro Ala Ala Gln Ala Lys Pro Gly Ala Lys
                155                 160                 165

agg gag cca ucu gcc ccg acu gaa ucu aca ucc caa gag aca ccu gaa      823
Arg Glu Pro Ser Ala Pro Thr Glu Ser Thr Ser Gln Glu Thr Pro Glu
            170                 175                 180

cag uca gac aag caa aca acg cca guc cag gga gcc aaa ucc aag cag      871
Gln Ser Asp Lys Gln Thr Thr Pro Val Gln Gly Ala Lys Ser Lys Gln
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gau ucu gag uca gaa uca gau gug gga uca ucu uca gac aca gau ucu     1015
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cca gcc acg aug ggu gga aug gug gcc cag gga gug aag cua ggc uuc     1063
Pro Ala Thr Met Gly Gly Met Val Ala Gln Gly Val Lys Leu Gly Phe
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aaa gga aaa ucu ggu uau aaa gug aug uca gga uac agu ggg acg ucg     1111
Lys Gly Lys Ser Gly Tyr Lys Val Met Ser Gly Tyr Ser Gly Thr Ser
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cca cau gag aaa acc agu gcu cgg aau cac aga cac uac cag gau aca     1159
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gcc uca agg cuc auc cac aac aug gac cug cgc aca aug aca cag ucg     1207
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gag cag gcg cug ggg cug gag ccg gcc cug ggc cgc cug cug ggu gug     1351
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        345                 350                 355

gcg cac cuc uuu gac cug gac cca gag aca ccg gcc aac ggg uac cgc     1399
Ala His Leu Phe Asp Leu Asp Pro Glu Thr Pro Ala Asn Gly Tyr Arg
    360                 365                 370

agc cua gug cac aca gcc cgc ugc ugc cug gcg cac cuc cug cac aaa     1447
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375                 380                 385                 390

ucc cgc uau gug gcc ucc aac cgc cgc agc auc uuc uuc cgc acc agc     1495
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                395                 400                 405

cac aac cug gcc gag cug gag gcc uac cug gcu gcc cuc acc cag cuc     1543
His Asn Leu Ala Glu Leu Glu Ala Tyr Leu Ala Ala Leu Thr Gln Leu
            410                 415                 420

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                475                 480                 485

auu ggg cug gug ucc uuc ggg gag cac uac aaa cgc aac gag aca ggc      1783
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            490                 495                 500

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gac gug cac uuc ugg aaa gcc uuc ugg aac auc acc gag aug gaa gug      1927
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cua ucg ucu cug gcc aac aug gca ucg gcc acc gug agg gua agc cgc      1975
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ccc acg cuc acg guc acc auc uca ccc cca cug gcc cac aca ggc ccu      2071
Pro Thr Leu Thr Val Thr Ile Ser Pro Pro Leu Ala His Thr Gly Pro
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gac agu gag gag cuc agc agc cug aua aag ucc aac ggc caa cgg agc      2167
Asp Ser Glu Glu Leu Ser Ser Leu Ile Lys Ser Asn Gly Gln Arg Ser
615                 620                 625                 630

cug gag cug ugg ccg cgc ccc cag cag gca ccc cgc ucg cgg ucc cug      2215
Leu Glu Leu Trp Pro Arg Pro Gln Gln Ala Pro Arg Ser Arg Ser Leu
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Ile Val His Phe His Gly Gly Gly Phe Val Ala Gln Thr Ser Arg Ser
            650                 655                 660

cac gag ccc uac cuc aag agc ugg gcc cag gag cug ggc gcc ccc auc      2311
His Glu Pro Tyr Leu Lys Ser Trp Ala Gln Glu Leu Gly Ala Pro Ile
        665                 670                 675

auc ucc auc gac uac ucc cug gcc ccu gag gcc ccc uuc ccc cgu gcg      2359
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cuc cuu ggc uca aca ggg gaa cga auc ugc cuu gcg ggg gac agu gca      2455
Leu Leu Gly Ser Thr Gly Glu Arg Ile Cys Leu Ala Gly Asp Ser Ala
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Gly Gly Asn Leu Cys Phe Thr Val Ala Leu Arg Ala Ala Ala Tyr Gly
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Val Arg Val Pro Asp Gly Ile Met Ala Ala Tyr Pro Ala Thr Met Leu
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cag ccu gcc gcc ucu ccc ucc cgc cug cug agc cuc aug gac ccc uug      2599
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Ser Gln Lys Met Ser Glu Pro Ile Ala Glu Pro Met Arg Arg Ser Val
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